



Phylogenetic Identification of Petroleum-Degrading Bacteria in Alaska Willow Soils

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Introduction

Background

- Certain plant species may promote growth and activity of pollutant-degrading microbes in the rhizosphere.
- Naphthalene is an aromatic component of petroleum fuels, which are common soil contaminants in Alaska.
- Willows are known to produce and release salicylate, an intermediate in the naphthalene degradation pathway that induces the expression of microbial naphthalene degradation genes.
- A previous pot study (McFarlin et al. in prep) tested the ability of *Salix alaxensis* (Alaskan willow) to rhizo-remediate diesel-contaminated soil.
- Willow growth treatments significantly decreased the concentration of diesel range organics in soil and increased the number of cultured diesel-degrading bacteria in comparison to unplanted controls.
- The effects of willow on the identity and diversity of diesel-degrading bacteria in this pot study are unknown.

Objectives

- 1) Use DNA sequencing to identify naphthalene-degrading bacteria in willow-planted and control (unplanted) soils at a phylogenetic level.
- 1) Compare the community profiles of bacterial naphthalene degraders between willow and control soil.

Methods

DNA Stable Isotope Probing

Links bacterial phylogeny with metabolic function; provides direct evidence of isotope-labeled substrate utilization

- 1) **Incubation:** Incubate soil samples with isotopically-labeled substrate (¹³C-naphthalene).
- 2) **Incorporation:** Organisms that utilize the labeled substrate for growth incorporate ¹³C into biomarker molecules (DNA).
- 3) **Detection:** ¹³C-labeled DNA is isolated and analyzed to determine taxonomic identity and diversity of degraders.

- McFarlin's experimental potted soil was incubated with ¹³C-naphthalene in triplicate microcosms for 0, 3, 7, and 14 days.
- DNA was extracted, subjected to density-gradient centrifugation, and fractionated to separate light (¹²C) and heavy (¹³C) DNA.
- qPCR of bacterial DNA was performed to locate heavy fractions containing ¹³C-labeled DNA.
- Terminal restriction fragment length polymorphism (T-RFLP) was performed on ¹³C-DNA to examine the diversity of naphthalene-utilizing community.

Results and Discussion

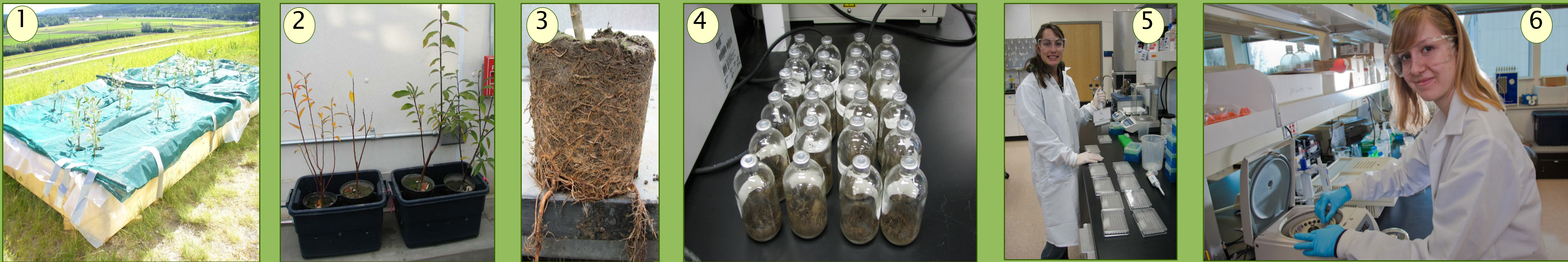


Figure 1: McFarlin's experimental pots (Figs. 1-3) and microcosms (Fig. 4), McFarlin's MPN experiment (Fig. 5), and Glover's SIP experiment (Fig. 6), photo credits - Todd Paris).

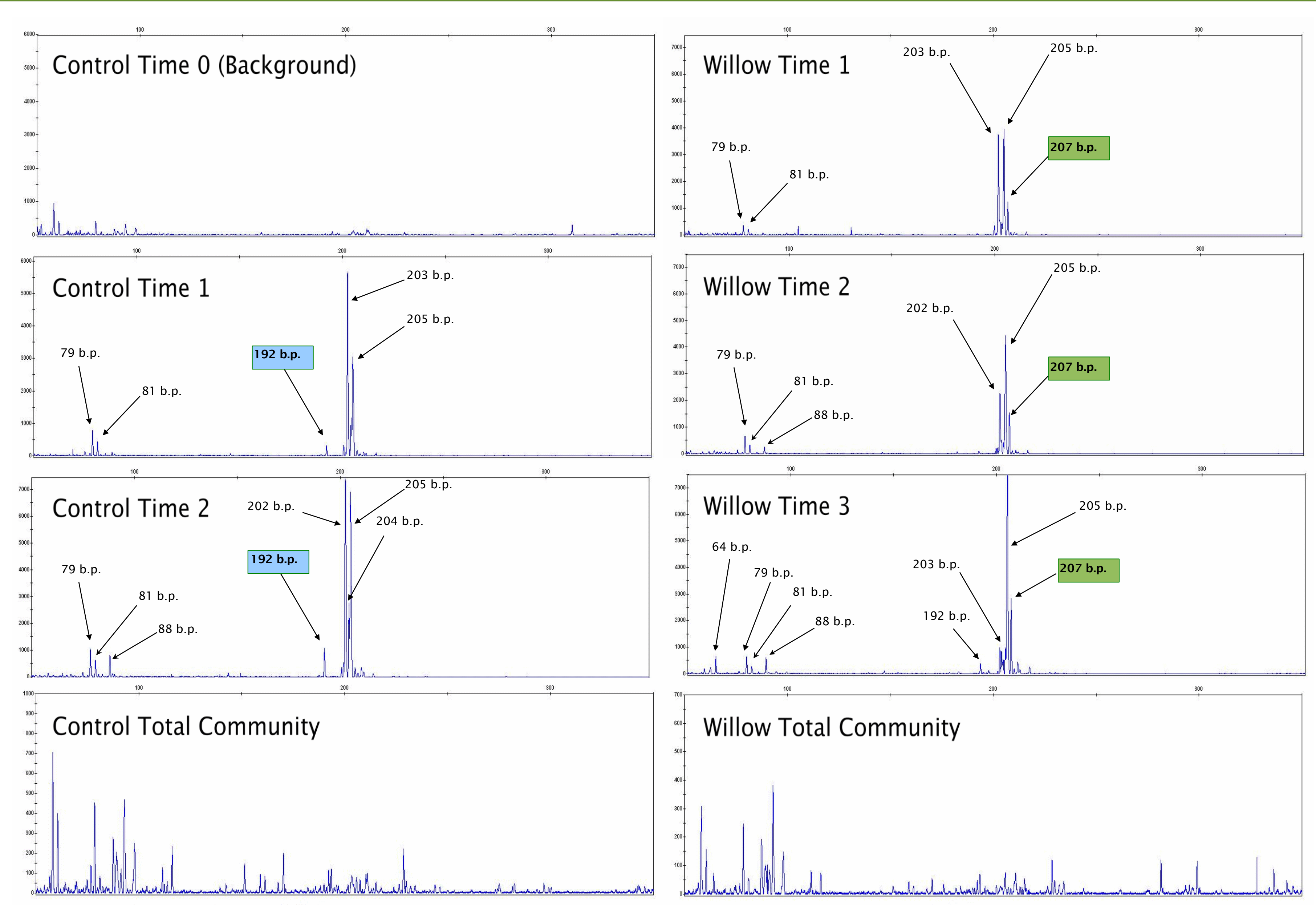


Figure 2: T-RFLP on bacterial ¹³C-labeled DNA, background total soil DNA, and total community fractions.

T-RFLP:

Diversity profiles of ¹³C control and willow bacterial communities showed many similarities, but also displayed a few key differences:

- Willow community showed 207 b.p.* as a large peak
- Control community showed 192 b.p. as a larger peak and contained more small peaks in 50-98 b.p. range

Total community samples showed diverse bacterial communities, but only a few small peaks at 201-207 b.p. where ¹³C-incubated samples produced large peaks.

- Bacteria utilizing labeled naphthalene may be present at relatively low abundance in the total soil community

* base pairs

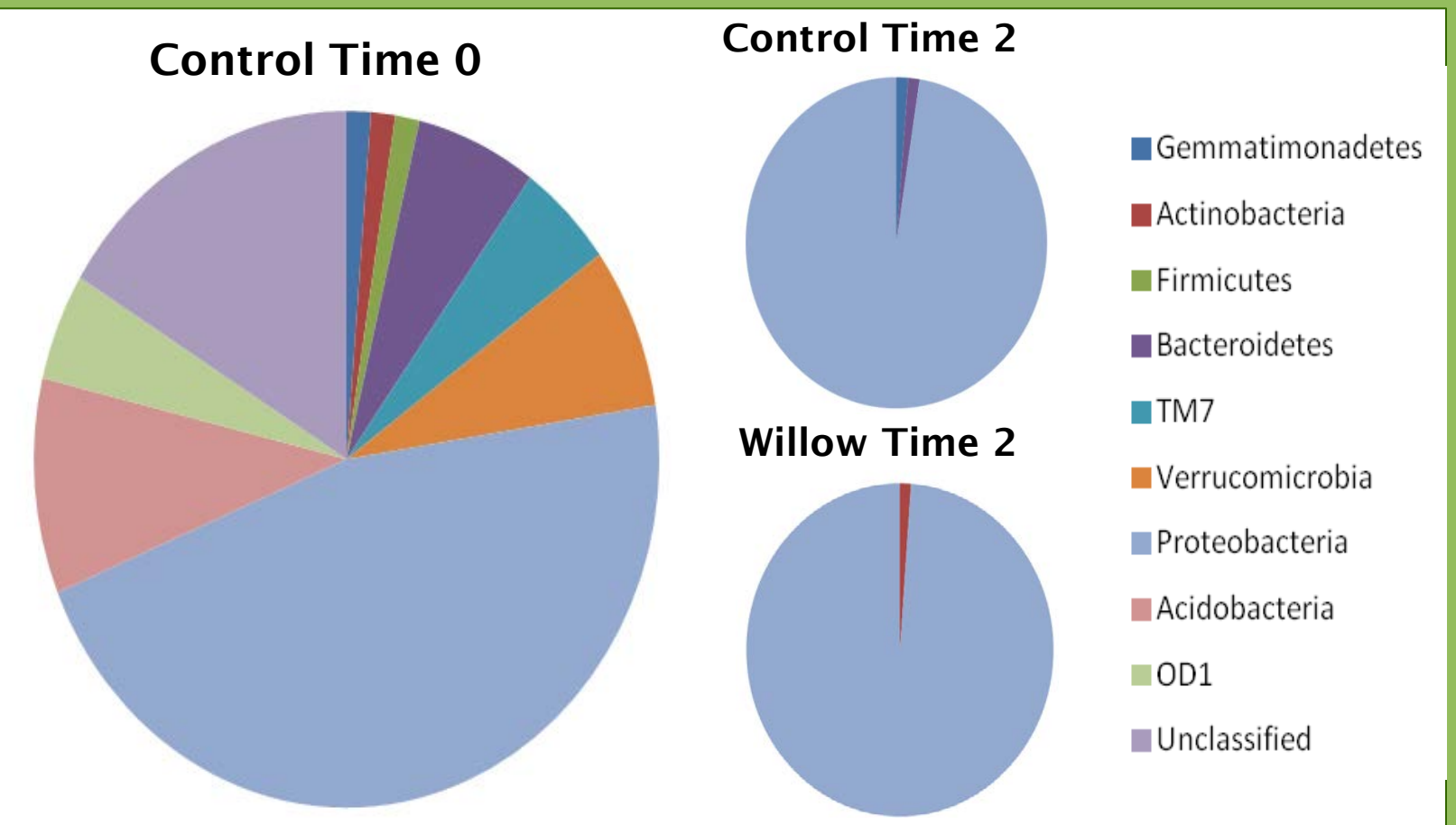


Figure 3: Proportions of bacterial phyla detected with Sanger sequencing in select ¹³C-incubated samples.

DNA Sequencing of bacterial 16S rRNA genes confirmed and elucidated the change in community composition after the addition of ¹³C-naphthalene.

- Control community is initially diverse with at least 10 different phyla present.
- *Proteobacteria* are enriched after ¹³C-naphthalene addition

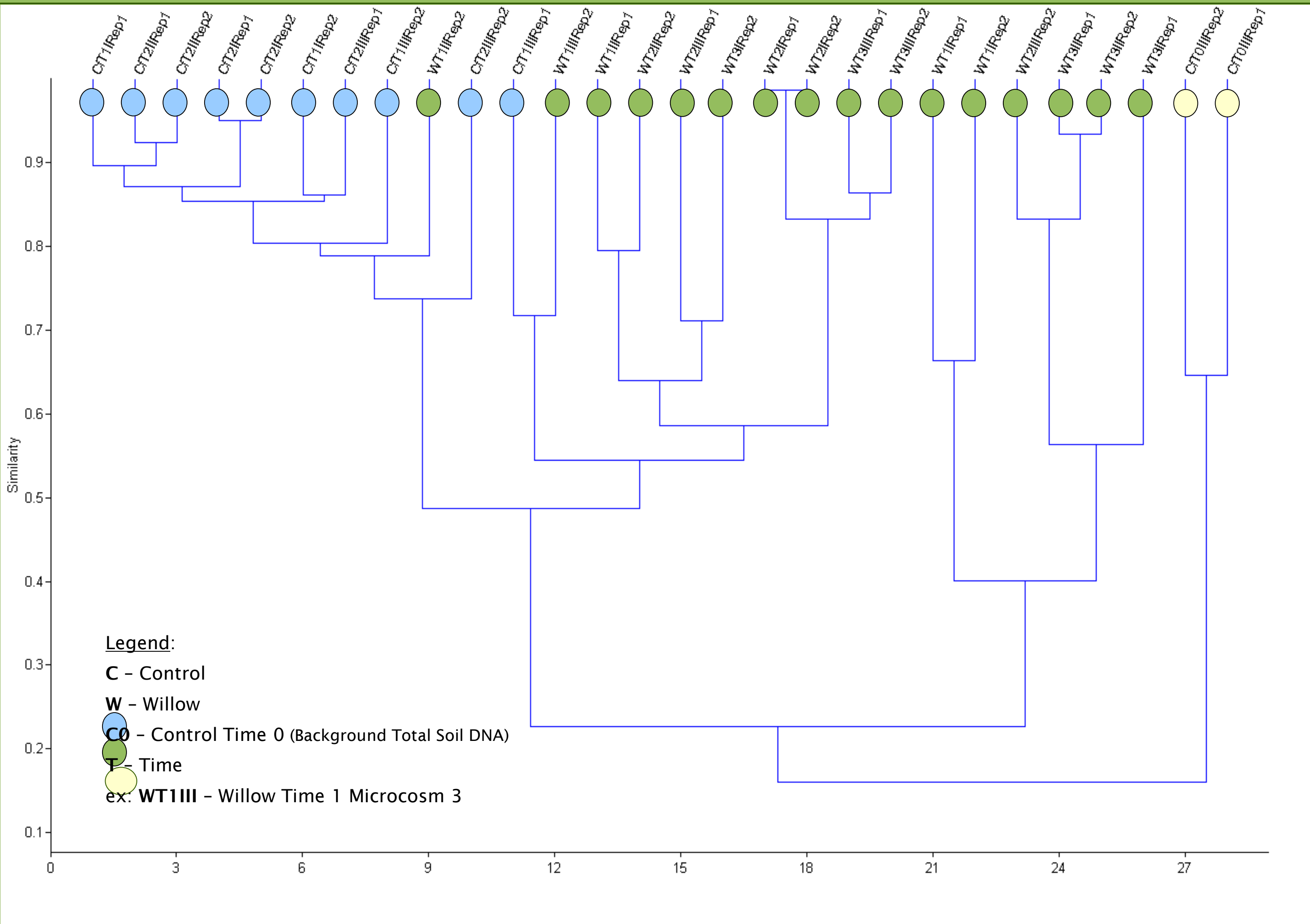


Figure 4: Cluster analysis of T-RFLP results from ¹³C-labeled DNA fractions with Bray-Curtis distance.

Cluster Analysis of ¹³C-labeled bacterial community profiles showed that:

- 1) control and willow samples often clustered separately
- 1) suggesting that willow presence altered the structure and/or composition of naphthalene-utilizing bacterial populations relative to that of unplanted soil.

Future Research

- Next-generation **pyrosequencing** is in progress to provide higher-resolution community profiles of the same control and willow samples with hundreds of thousands of DNA sequences
- This study examined unfertilized willow treatments and controls. Fertilized willow and control soils have also been incubated and will be analyzed to investigate how nutrient addition impacts the identity and diversity of bacterial naphthalene degraders.

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